Structure, Organization, and Expression of the lct Gene for Lacticin 481, a Novel Lantibiotic Produced by Lactococcus lactis

Piard, Jean-Christophe; Kuipers, Oscar P.; Rollema, Harry S.; Desmazeaud, Michel J.; Vos, Willem M. de

Published in:
The Journal of Biological Chemistry

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
1993

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment.

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.
The structural gene for the lactococcal lantibiotic lacticin 481 (lct) has been identified and cloned using a degenerated 20-mer DNA oligonucleotide based on the amino-terminal 7 amino acid residues of the purified protein. The transcription of the lct gene was analyzed, and its promoter was mapped. DNA sequence analysis of the lct gene revealed an open reading frame encoding a peptide of 51 amino acids. Comparison of its deduced amino acid sequence with the amino-terminal sequence and the amino acid composition of lacticin 481 indicates that the 51-residue peptide is prelacticin 481, containing a 27-residue carboxyl-terminal propeptide and a 24-residue amino-terminal leader peptide which lacks the properties of a typical signal sequence and which is significantly different from the leaders of other lantibiotics. The predicted amino acid sequence of prolacticin 481 contains 3 cysteines, 2 serines, and 2 threonines which were not detectable in amino acid analyses of mature lacticin 481. Based on these results and on characterization by two-dimensional NMR techniques, a structural model is proposed in which 2 cysteine residues are involved in lanthionine and one in $\beta$-methylanthionine formation, and a 4th threonine residue is dehydrated. This model predicts a molecular mass for lacticin 481 of 2,901, which is in excellent agreement with that obtained from mass spectrometry.

Lactic acid bacteria are Gram-positive bacteria that are frequently able to produce proteins with antimicrobial activity, designated bacteriocins (Piard and Desmazeaud, 1992). The bacteriocins of lactic acid bacteria which have been biochemically characterized include 2 types of peptides. Those containing the common 20 amino acids residues, such as lactacin F produced by Lactobacillus acidophilus (Muriana and Klaenhammer, 1991a, 1991b), lactococcin A from Lactococcus lactis (Holo et al., 1991; van Belkum et al., 1991), and leuconocin A-UAL 187 from Leuconostoc gelidum (Hastings et al., 1991), containing 57, 54, and 37 amino acids, respectively. A second class of peptides, called lantibiotics, is characterized by the presence of dehydrated amino acids (dehydroalanine and dehydrobutyrine) and thioether amino acids lanthionine and $\beta$-methylanthionine (Schnell et al., 1988). The lantibiotics nisin A (Hurst, 1981; Buchman et al., 1988), nisin Z (Mulders et al., 1991), and lacticin 481 (Piard et al., 1990, 1992), are produced by L. lactis, and lactocin S, by Lactoccillus sake (Méryptet et al., 1991). Lantibiotics have also been isolated from other Gram-positive organisms, such as Bacillus subtilis (subtilin), Staphylococcus epidermidis (epidermin and Pep6), and Staphylococcus gallinarum (gallidermin) (Jung, 1991). In contrast to most antibiotics, the lantibiotics are synthesized via a ribosomal pathway as prepeptides which are subsequently modified. The serine and threonine residues are dehydrated to dehydroamino acids which can react with the thiol group of cysteine to form the thioether bridges of lanthionine or $\beta$-methylanthionine, respectively. Nisin is the best characterized lantibiotic, and its spatial structure has been studied by two-dimensional NMR (van de Ven et al., 1991; Lian et al., 1992). However, extensive structure-function studies are necessary to understand the molecular basis of lantibiotic action and to be able to construct mutants with an improved spectrum of activity and physico-chemical properties. To achieve this, two approaches are presently followed. One is to generate by random or site-directed mutagenesis new lantibiotics and study their properties. The other is to characterize the structure and action of naturally occurring lantibiotics. The feasibility of both approaches has been shown in studies of nisin, by engineering dehydrated and other residues (Kuipers et al., 1992), and analyzing the biological activities of the two natural variants nisin A and nisin Z (de Vos et al., 1993). Here we continue with the fast approach and focus on the lantibiotic lacticin 481, which is a broad spectrum bacteriocin exhibiting bactericidal activity against a wide range of lactic acid bacteria and against Clostridium tyrobutyricum (Piard et al., 1990). We describe the structure, organization, and transcription of the lct gene encoding lacticin 481. In addition, we propose a structural model for lacticin 481 based on the deduced amino acid sequence of prelacticin 481, its amino acid composition and characterization by two-dimensional NMR spectroscopy.

**EXPERIMENTAL PROCEDURES**

**Bacterial Strains, Plasmids, and Media—**Lacticin 481-producing L. lactis subsp. lactis (further designated as L. lactis) CNRZ 481 was obtained from the CNRZ culture collection (INRA, Jouy-en-Josas, France). The nisin producer L. lactis NIZO R5 harboring the niaA encoding transposon Tn5276 (Rausch and de Vos, 1992) was from NIZO. A lacticin 481-producing (Lct*) transconjugant, L. lactis JC17, was obtained by conjugal matings between L. lactis 481 (Lct*) and L. lactis IL1441 (Lct*). Microbial Genetics Laboratory, INRA as described elsewhere. Escherichia coli JM83 (Vieira and Messing, 1982) and TG1 (Gibson, 1984) were used as hosts in cloning experiments.
cilline (100 pg/ml) and erythromycin (5 pg/ml) were added. Isopropyl-
containing 0.5% glucose at 30 °C. If appropriate, antibiotics carbeni-
sis-Total DNA from galactoside were used at 0.025%.

FIG. 1. Single-stranded DNA sequence of the region containing iet
gene and deduced amino acid sequence. The processing site in prolec-
ticin 481 is indicated (Δ). The promoter consensus sequences (~35, ~10) and po-
tential ribosome binding site (RBS) are underlined. The transcription start site of
lct is indicated by an arrow. Termination codons are underlined. The hori-
zontal dashed arrows below the nucleo-
tide sequence indicate inverted repeats. Identical and functionally related amino
acids between the deduced residues from the upstream open reading frame and IS4 transposes are indicated by an as-
terisk and a black dot, respectively.

For expression studies L. lactis MG1614 (Gasson, 1983), IL1441 and
NIZO R520 (Rauch et al., 1991) were utilized. Plasmids pUC19 (Vieira
and Messing, 1982) and pIL253 (Simon and Chopin, 1988) were used
as cloning vectors in E. coli and L. lactis, respectively.

E. coli strains were grown in L-broth (Sambrook et al., 1989) at
37 °C and L. lactis strains in M17 broth (Terzaghi and Sandine, 1975)
containing 0.5% glucose at 30 °C. If appropriate, antibiotics carbeni-
cillin (100 μg/ml) and erythromycin (5 μg/ml) were added. Isopropyl-
1-thio-β-D-galactopyanoside and 5-bromo-4-chloro-3-indolyl
β-D-galactoside were used at 0.05%.

DNA Methodology, Southern Hybridization, and Sequence Analy-
sis—Total DNA from L. lactis was isolated as described by Rauch
de Vos (1992). Plasmid DNA was extracted from E. coli by the
alkaline lysis method (Sambrook et al., 1989). For plasmid extraction in
L. lactis, this procedure was slightly modified as described by de
Vos and Gasson (1989). Subsequent manipulations in vitro and in E.
coli were performed as described (Sambrook et al., 1989). L. lactis
was transformed by electroporation using a GenePulser (Bio-Rad) (VOS
et al., 1989). For Southern hybridization, DNA was electrophoresed
in an agarose gel, deparaffinized for 20 min in 0.25 M HCl, and blotted
for 1 h onto a nylon GeneScreen Plus membrane (Du Pont) using a
blotting device (VacuGene, Pharmacia LKB Biotechnology Inc.) and
1 M NaOH as a transfer buffer. Colony hybridization was performed
with the same type of membrane (Sambrook et al., 1989). DNA probes
were end labeled with [γ-32P]ATP and T4 polynucleotide kinase. A
degenerated 20-mer, P25 (5'-ATNACNCCCA/GCTNCNCCCT/T
CJT-3') was synthesized on a Biosearch Cyclone DNA Synthesizer
(New Brunswick Scientific) and used to identify the lct gene in both
Southern and colony blot hybridizations. Hybridizations and washing
were performed at 52 and 37 °C, respectively.

DNA sequencing was performed by the dideoxy chain termination
method (Sanger et al., 1977) using M13 universal primers and primers
P28 (5'-CTTGAAGAGAGATTTAAAAGAG-3') and P29 (5'-GTATT-
TACTTGCTGCTC-3'), complementary to position 844-824 and
946-963 of the lct gene, respectively (see Fig. 1). Computer analysis
of DNA and amino acid sequences was performed with the programs
of PC/GENE (Genetech). DNA probes

DNA Isolation, Northern Blot Analysis, and Primer Extension Studies—L. lactis strains were grown to A600 nm = 0.6. Cells
were pelleted, resuspended in 0.5 ml of TE (10 mM Tris-HCl, pH 7.5, 1
mM EDTA) and kept on ice. Subsequently, the suspension was
transferred to a microcentrifuge tube containing 0.6 g of zirconium
beads (Biospec Products), 0.17 g of 4% Macaloid (Rheox, for
suspension see below), 0.5 ml of phenol, pH 7.5, and 0.05 ml of 10%
sodium dodecyl sulfate. The tubes were shaken at maximal setting
in a Biospec homogenizer at room temperature for 3
min. The gel was allowed to cool, washed with TE, centrifuged, and
resuspended before each use.

For Northern blot analysis, RNA was glyoxylated, fractionated on
12% (15 cm) polyacrylamide gels, transferred to a microcentrifuge tube containing 0.6 g of zirconium
beads (Biospec Products), 0.17 g of 4% Macaloid (Rheox, for
suspension see below), 0.5 ml of phenol, pH 7.5, and 0.05 ml of 10%
sodium dodecyl sulfate. The tubes were shaken at maximal setting
in a Biospec homogenizer at room temperature for 3
min. The gel was allowed to cool, washed with TE, centrifuged, and
resuspended before each use.

Macaloid 4% was prepared as follows. Two grams of Macaloid was
suspended in 100 ml of TE, boiled for 5 min, and sonicated
with a sterilized probe sonicator (Heat Systems) until a gel formed (about
1 min). The gel was allowed to cool, washed with TE, centrifuged, and
separated from the supernatant. This allowed us to recover about 50
ml of gel.

For Northern blot analysis, RNA was glyoxylated, fractionated on
a 1.0% agarose gel, and blotted and hybridized as described previously

1 H. Raya, personal communication.
Structure of the *L. lactis* *lct* Gene for Lacticin 481

(Reprinted from the Journal of Biological Chemistry, 16363, van Rooijen and de Vos, 1990.)

**Table I**

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>No. of residues/molecule in Lacticin 481</th>
<th>Most probable no. of processed residues</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asx</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Cys</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Glx</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Gly</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>His</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Ile</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Lan + β-CH3Lan</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Lys</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Met</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Phe</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Ser</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Thr</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Trp</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Val</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

Total 23

Lan, lanthionine; β-CH3Lan, β-methylthionine.

* Obtained from amino acid analyses.

† Deduced from the DNA sequence of *lct*.

**Fig. 2.** Northern blot analysis of RNA isolated from lane 1, *L. lactis* CNRZ 481 (*Lct*); lane 2, *L. lactis* JC17 (*Lct*); lane 3, *L. lactis* IL1441 (*Lct*). Hybridization was performed with a single-stranded probe for the *lct* gene and upstream region (see “Experimental Procedures”). The size of the transcripts is indicated on the left, in bases (b).

**Fig. 3.** Primer extension products of RNA transcribed from *lct*. The relevant DNA sequence (complementary to *lct* sequence) is indicated, and the determined transcription start site is marked by an asterisk. Lanes A, C, G, T are sequencing reactions carried out as described under “Experimental Procedures”; lanes 1 and 2, *L. lactis* CNRZ 481 (*Lct*) with and without primer, respectively; lanes 3 and 4, *L. lactis* JC17 (*Lct*) with and without primer, respectively; lane 5, *L. lactis* IL1441 (*Lct*) with primer.

**Amino Acid Analysis of Lacticin 481**—Fifteen liters of lacticin 481-containing supernatant was purified according to the previously reported procedure (Piard et al., 1992) except that the gel filtration step was omitted. Preparative reverse phase chromatography was performed using C18 Sep-Pak cartridges equilibrated with 25 mM ammonium acetate. Lacticin 481 activity was eluted with 50% 2-propanol in 20 mM ammonium acetate. Additional and final purification was achieved by reverse-phase high performance liquid chromatography (Waters, model 510) using a 250 x 20-mm Hi-Pore RP-318 (Bio-Rad) column at 30 °C. Running buffers (10 ml/min) were 10% acetonitrile in 25 mM ammonium acetate (buffer A) and 60% acetonitrile in 20 mM ammonium acetate (buffer B). A linear gradient of 55% buffer A plus 45% buffer B, to 45% buffer A plus 55% buffer B run within 35 min was used. Absorbance was monitored at 220 nm, and a data acquisition system (Waters Maxima 820) was used. The active eluting fractions were pooled, and ammonium acetate was removed by consecutive washes with distilled water and freeze-drying.

**Amino Acid Analysis of Lacticin 481**—Pure lacticin 481 was hydrolyzed for 24 and 96 h in 6 M HCl at 110 °C; the 96-h hydrolysis was used for the complete hydrolysis of Val-Ile bonds. The hydrolysates were concentrated by lyophilization, dissolved in 0.2 mM sodium citrate, pH 2.2, and analyzed on an amino acid analyzer (LKB, type 4151). The sum of the lanthionine and β-methylthionine residues was determined using D,L-lanthionine as a standard.

**Mass Spectrometry**—Lacticin 481 was analyzed as described by van Dorsselaer et al. (1990) using a VG Biotech BioQ mass spectrometer (VG Biotech Ltd., Altrincham, U. K.) consisting of an electrostatic spray ion source operating at atmospheric pressure, followed by a quadrupole mass analyzer with a mass range of 4,000. The electrospray potential was about 4,000 V. The voltage of the extracting cone was adjusted to 100–200 V. Lacticin 481-containing samples were prepared in methanolic acid, 99% (v/v), and 10 μl was introduced into the ion source at a flow rate of 3 μl min⁻¹. Results were processed using a data system and quoted as a mean molecular mass derived from several multiply charged ion peaks plus the standard deviation.

**NMR Spectroscopy**—One- and two-dimensional NMR spectra were taken on Bruker AM400 and AM600 spectrometers operating at 400.13 and 600.13 MHz, respectively. The spectra were referenced to
The resulting recombinant transformants were screened by position of lacticin 481 (see below) revealed that the 51-kilobase copy; NOESY, nuclear Overhauser enhancement spectroscopy; L. subcloned in M13mp18 and M13mp19 for sequencing. EcoRI-linearized pUC19, and introduced into and Southern hybridization of pLCT8 revealed that the otide P25. One of the colonies showed a reproducible strong colony blot and Southern hybridization using the oligonucleotide showed the amino-terminal sequence and the amino acid composition. The calculated molecular mass for prolacticin 481 is 2973 Da. A potential Shine-Dalgarno sequence (GGAG) is located 7 base pairs upstream of the ATG start codon of the lct gene (ΔG value of complementarity to the L. lactis 3' 16 S rRNA sequence was -9.4 kcal mol⁻¹). Twenty-seven base pairs downstream of the lct stop codon an inverted repeat was identified which could act as a rho-independent terminator with a ΔG value of -20.6 kcal mol⁻¹ (D'Aubenton Carafa et al., 1990).

Analysis of the DNA sequence upstream of the lct gene showed the start of a putative open reading frame. A data base search revealed that the deduced protein sequence had significant similarity (26% identity within a stretch of 88 amino acids) with the transposase from the E. coli IS4 insertion element (Fig. 1). Analysis of the DNA sequence downstream of the lct gene showed the start of a putative open reading frame with a GTG start codon preceded by a potential ribosome binding site (AAGGA). However, no clear promoter consensus sequence was identified. Data base searching did not show similarity with other DNA sequences including those flanking structural genes of other lantibiotics.

**RESULTS**

**Isolation and Sequence Analysis of Lacticin 481 Structural Gene, lct, and Its Flanking Regions**—Southern blots of EcoRI-digested total DNA from L. lactis 481 (Lct⁺), JC17 (Lct⁺), and IL1441 (Lct⁻) were hybridized with the degenerated P25 oligonucleotide based on the amino-terminal amino acid sequence of lacticin 481. Hybridization was found with a 9.5-kb fragment of L. lactis 481 DNA and a 4.5-kb fragment DNA of L. lactis JC17 (data not shown). In contrast, no hybridization signal was detected with DNA from L. lactis IL1441. The 4.5-kb EcoRI fragments of L. lactis JC17 were isolated, ligated to EcoRI-linearized pUC19, and introduced into E. coli JM83. The resulting recombinant transformants were screened by colony blot and Southern hybridization using the oligonucleotide P25. One of the colonies showed a reproducible strong signal and contained the plasmid pLCTS. Restriction analysis and Southern hybridization of pLCTS revealed that the lct gene was present on a 1.3-kb BamHI-HindIII subfragment that was subcloned in M13mp18 and M13mp19 for sequencing.

Analysis of the 1.3-kb DNA fragment sequence showed an open reading frame that could encode a peptide of 51 amino acids (Fig. 1). Comparison of the deduced amino acid sequence with the amino-terminal sequence and the amino acid composition of lacticin 481 (see below) revealed that the 51-residue peptide is prelacticin 481 consisting of a 27-residue carboxyl-terminal propeptide and of a 24-residue amino-terminal extension.

The transcription start site of lct in strains L. lactis 481 and JC17 was determined by primer extension mapping of RNA, using oligonucleotide P28 as primer. The same sized primer extension product was detected in both lacticin 481 producer strains.

---

The abbreviations used are: TOCSY, total correlated spectroscopy; NOESY, nuclear Overhauser enhancement spectroscopy; kb, kilobase(s).
Structure of the L. lactis lct Gene for Lacticin 481

PLCT9 or PLCT10 did not result in lacticin 481 production. Yields of nisin-producing strains (Kuipers et al., 1992) located 6 base pairs upstream of the transcription initiation site and consisting of the canonical promoter and shows that increasing the copy number of the lct gene results in increased production of lacticin 481. Transfection of L. lactis 481 harboring PLCT9 or PLCT10 produced similar amounts of lacticin 481, which indicates that the systems allowing nisin maturation and secretion are not efficient for the processing of lacticin 481.

Amino Acid Content and Mass Spectrometry of Lacticin 481—Prerequisite to prediction of the structure of lacticin 481 is an accurate determination of the amino acid composition. The amino acid composition deduced from the DNA sequence differed from that reported previously (Piard et al., 1992). We therefore performed extended acid hydrolysis of lacticin 481. These analyses resolved additional isoleucine and valine residues and two additional lanthionine or β-methylthionine residues that had not been detected in previous analyses (Table I). Twenty-three amino acids are identified in mature lacticin 481. Any dehydrated amino acid residues would not be detected in these analyses. Lacticin 481 contains a relatively high content of glycine (13%) and a high proportion of hydrophobic or apolar amino acids. As predicted by PC/GENE, prolacticin 481 has an isoelectric point of 7.1 (7.7 if we consider that the 3 cysteines form thionionine rings) because of the presence of 2 histidines, 1 lysine, and 1 glutamic acid residue.

Mass spectral measurements of lacticin 481 using electrospray ionization are shown in Fig. 4. From the series of multiple charged ions an average molecular mass of m/z = 2,901.14 ± 0.28 Da was found.

NMR Spectroscopy.—In the one-dimensional NMR spectrum of lacticin 481 (Fig. 5) some unusual features were observed. An appreciable number of amide resonances, particularly those from the residues which form part of the three cyclic structure (see below), show a more or less severe line broadening. An increase in temperature reduces this line broadening (Fig. 5, a and b). On the other hand a comparison of 400 and 600 MHz NMR spectra (Fig. 5, a and c) does not show the improvement in resolution expected at the higher spectrometer frequency. These observations indicate the presence of chemical exchange: i.e. the molecule is exchanging between two (or more) states (most probably conformational states).

Using standard techniques (amino acid pattern recognition from TOCSY spectra and sequential assignment based on NOESY data) an almost complete assignment of the NMR spectrum of lacticin 481 was achieved (Table II). The NOESY spectrum showed sequential connectivities (NH-NH+, CαH-NH+, NH+, or other) for the segments 3–10, 11–14, 15–19, and 22–26. The resonances of Lys1, Gln2, Phe4, and Ser7 were assigned by elimination. In the case of Asp11, Phe23, and Phe28 a complete assignment was not possible, most probably because of a severe broadening of their NH resonances.

The β-methylthionine Abu-S-Ala14 was readily identified by its relatively strong β-β and β-γ contacts in the NOESY spectrum. However, the β-β contacts of the remaining two thionionines were not observed, leaving two possibilities for the strucure of lacticin 481 i.e., thioether bridges between residues 11 and 25, and 18 and 26 or between residues 11 and 26, and 18 and 25.

The NMR data showed unequivocally that a dehydrobutyrine residue is located at position 24. From the higher intensity of the NH-β NOE as compared with that of the NH-γ cross-peak, it can be concluded that the dehydrobutyrine residue has the Z conformation (i.e. -C=H directed toward the NH group), which has also been observed for several dehydrobutyrine residues in nisin and nisin mutants (Chan et al., 1989; Kuipers et al., 1992).

Discussion

DNA sequence analysis of the lct gene reveals lacticin 481 as a new lactococcal lantibiotic that is ribosomally synthesized...
pared with the amino acid content of lacticin 481 shows that it shares the same profile as that of other prelantibiotics (Jung, 1991). The deduced amino acid composition of prolacticin 481 contains a hydrophobic carboxyl-terminal propeptide (prolacticin 481) and a rather hydrophilic amino-terminal extension. The deduced amino acid composition of prolacticin 481 compared with the amino acid content of lacticin 481 shows that as a prepeptide of 51 amino acids. The lct gene appears to be organized as single transcriptional unit since the size of the transcript (280 bases) corresponds to that predicted from the DNA-deduced protein sequence are not found in mature lacticin 481 (Table I). On the other hand, 3 additional \((\beta\text{-methyl})\)lanthionine residues were detected in mature lacticin 481 (Table I). On the other hand, 3 additional \((\beta\text{-methyl})\)lanthionine residues were detected in mature lacticin 481 and with Cys\textsubscript{3} and Cys\textsubscript{14}, in the other case (Fig. 6B). In both models, the rigidity of lacticin 481 would be high because of the presence of cyclic structures in the peptide chain of lacticin 481. Such rigidity, in the absence of other secondary structural elements, could be important in the ability of lantibiotics to form pores in membranes.

Structure of Lacticin 481—Analysis of the hydropathy of the 51 residue prelacticin 481 shows that it shares the same profile as that of other prelantibiotics (Jung, 1991) i.e., it contains a hydrophobic carboxyl-terminal propeptide (prolacticin 481) and a rather hydrophilic amino-terminal extension. The deduced amino acid composition of prolacticin 481 compared with the amino acid content of lacticin 481 shows that 2 serine, 2 threonine, and 3 cysteine residues that should be present based on the DNA-deduced protein sequence are not found in mature lacticin 481 (Table I). On the other hand, 3 additional \((\beta\text{-methyl})\)lanthionine residues were detected in lacticin 481. We consider it likely that of the 4 additional serine and threonine residues detected in prolacticin 481 are dehydrated and condensed with the cysteines to form the 3 \((\beta\text{-methyl})\)lanthionine residues of mature lacticin 481 and that the 4th additional serine or threonine is a dehydrated residue. This structural model for lacticin 481 predicts a molecular mass of 2,901 Da, which is in excellent agreement with that estimated by electrospray mass spectrometry. The NMR data show clearly the presence of a thioether bridge between residues 9 and 14 and a dehydrobutyryl residue at position 24. The presence of two lanthionines could be inferred from the NMR spectra, but the positions of the two thioether bridges could not be established. Therefore, two structural models can be proposed for lacticin 481, in which Ser\textsubscript{14} and Ser\textsubscript{18} are, respectively, involved in lanthionine formation with Cys\textsubscript{26} and Cys\textsubscript{37} in one case (Fig. 6A) and with Cys\textsubscript{26} and Cys\textsubscript{45}, in the other case (Fig. 6B). In both models, the rigidity of lacticin 481 would be high because of the presence of cyclic structures in the peptide chain of lacticin 481. Such rigidity, in the absence of other secondary structural elements, could be important in the ability of lantibiotics to form pores in membranes.

Since in lacticin 481 the lanthionine moieties originating from dehydrated residues are situated on the amino-terminal side of their respective "cysteine" partners, lacticin 481 might be classified as a type A lantibiotic (Jung, 1991). However, the low net charge of lacticin 481 (+1) and the intramolecular
TABLE III  
Alignment of prolantibiotics

<table>
<thead>
<tr>
<th>Lantibiotic</th>
<th>Prolantibiotic sequence*</th>
</tr>
</thead>
<tbody>
<tr>
<td>nisin A</td>
<td>ITESIGCTPGCAKTGLNMTACTCHCSIHVSK</td>
</tr>
<tr>
<td>nisin Z</td>
<td>ITESIGCTPGCAKTGLNMTACTCNCSIHVSK</td>
</tr>
<tr>
<td>subtilin</td>
<td>WKBESIGCTPGCAVTGALQTOPLQTLCNC--KISK</td>
</tr>
<tr>
<td>epidermin</td>
<td>IAQKPCZTPGCAKRTGSFNPSYCC</td>
</tr>
<tr>
<td>gallidermin</td>
<td>IAGPAIRALSSQVOCQRLATRLPTVSCGKNGGK</td>
</tr>
<tr>
<td>pep5</td>
<td>GKNQVFPTILHEHLNAPLA. . . . . . .</td>
</tr>
<tr>
<td>lacticin 481</td>
<td>KGCSGVIHTISHECHNNSWQFPTCCS</td>
</tr>
</tbody>
</table>

* The processed residues are underlined; identical amino acids are boxed; for streptococcin A-FF22, the sequence reported is that of the mature peptide obtained from Edman degradation that stopped at position 23; dots indicate blank cycles. Sequence references: 1, Buchman et al. (1988); 2, Mulders et al. (1991); 3, Banerjee and Hansen (1988); 4, Allgaier et al. (1986); 5, Kellner et al. (1988); 6, Kellner et al. (1991); 7, Jack and Tagg (1991).

TABLE IV  
Alignment of lacticin 481 leader sequence with those of lantibiotic-type and non-lantibiotic bacteriocins

<table>
<thead>
<tr>
<th>Bacteriocin*</th>
<th>Leader sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lantibiotics</td>
<td></td>
</tr>
<tr>
<td>nisin A and Z1, 2</td>
<td>M STK- - D FNLDDL- VSVSKK- DSGAPR1</td>
</tr>
<tr>
<td>subtilin</td>
<td>MSKPDPPDLDV- VKVSQ- DSKITPQW</td>
</tr>
<tr>
<td>epidermin4</td>
<td>MEAVKEKNDLPFNLDVVKVNAKESNDGAEPR1</td>
</tr>
<tr>
<td>gallidermin5</td>
<td>MEAVKEKNELFDLDLVKVAKESNDGAEPR1</td>
</tr>
<tr>
<td>pep5</td>
<td>MKNNKNLFDLBEIKKKTSMNTDELEPQOT</td>
</tr>
<tr>
<td>lacticin 481</td>
<td>MKEQN-SFPNL-LQ-EVTESELDDLILGA2K</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Other bacteriocins</th>
<th>Leader sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>lactococcin A7</td>
<td>MKNQLNPFNIIV- - SDEELSEANGK</td>
</tr>
<tr>
<td>lactococcin B8</td>
<td>MKNQLNPFIIV- - SDEELAEVNGSS</td>
</tr>
<tr>
<td>lactococcin Ma9</td>
<td>MKNQLNPFLIL- - SDEBLQINGG1</td>
</tr>
<tr>
<td>lactocin F10</td>
<td>MK-Q- - FPNL- - SHKDLAVVGCGR</td>
</tr>
<tr>
<td>pediocin PA-111</td>
<td>MK- - KIEKL- - TEKEMAnIIGK</td>
</tr>
<tr>
<td>leucocin A12</td>
<td>MNNMKTPSYEQL- - DNSALEOQVGGK</td>
</tr>
<tr>
<td>[lacticin 481]</td>
<td>MKEQNSFPNLLOQEVTESELDDLILGA2K</td>
</tr>
</tbody>
</table>

* 1, 2, 3, 4, 5, 6, same references as in Table III; 7, van Belkum et al. (1991) and Holo et al. (1991); 8, van Belkum et al. (1992); 9, van Belkum et al. (1991); 10, Muziana and Klaenhammer (1991b); 11, Marrug et al. (1992); 12, Hastings et al. (1991).

distance between the ring-forming residues are more reminiscent of type B lantibiotics. Comparison of prolacticin 481 with other type A prolantibiotic sequences is shown in Table III. Prolacticin 481 appears significantly different from those prolantibiotics in that the homology in the position of the processed residues in pronisin, prosubtilin, proepidermin, progallidermin, and proppep5 was not found in prolacticin 481. However, a high level of sequence similarity was observed between lactacin 481 and streptococcin A-FF22 from Staphylococcus pyogenes that contains three lanthionine or β-methylthionine residues (41% identity). In addition, the positions of putative processed residues in lactacin 481 correspond to those of the residues blank cycles after Edman degradation in streptococcin A-FF22 (Table III). Since Edman degradation provides blank cycles for lanthionine or β-methylthionine residues and stops when it meets a dehydroamino acid, the residues in positions 8, 10, 13, and 17 in streptococcin A-FF22 might be involved in lanthionine or β-methylthionine formation, and residue in position 23 might be a dehydroamino acid. Therefore, lactacin 481 and streptococcin A-FF22 appear to share a similar structure. Further comparison awaits DNA sequence or structural data on A-FF22. In previous studies on lantibiotics, it was suggested that there was a common lantibiotic ancestor which would have diverged in different organisms leading to the occurrence of various structural variants (Buchman et al., 1988). Although this hypothesis appears to be justified with respect to the homology between nisin A, nisin Z, subtilin, epidermin, and gallidermin, it seems that lactacin 481 and...
streptococcin A-FF22 form a new class of the type A lantibiotics.

**Leader Sequence of Lactocin 481**—Typical signal sequences have a hydrophobic core and conform to the −1, −3 rule of von Heijne (1988). Analysis of the amino-terminal extension of prelactocin 481 shows that it lacks these properties. Alignment of the 21-residue amino-terminal extension from prelactocin 481 with other leader peptides in lantibiotics (Table IV) reveals no homologies, apart from the F(N/D)L consensus sequence. Unexpectedly, the amino-terminal extension of prelactocin 481 shows significant similarity to leader peptides of non-lantibiotics as well as a higher similarity to those of non-lantibiotics also points to other possible functions, such as interaction with the translocation and processing proteins, leading to secretion of active lactocin 481.

**Acknowledgments**—We are grateful to Alain van Dorselaer and to Odile Sorokine for mass spectrometry measurements, to Peter van Roojen for amino acid analyses, to Roland Sienz for helpful discussions, and to Patrick Regent for preparing the photographs. We are indebted to Alexandra Guss for help in the revision of the manuscript.

**REFERENCES**


